

An analysis of false positive reactions occurring with the Captia Syph G EIA

J Ross, A Moyes, H Young, A McMillan

Abstract

Aim—The Captia Syph G enzyme immuno assay (EIA) offers the potential for the rapid automated detection of syphilis antibodies. This study was designed to assess the role of other sexually transmitted diseases (STDs) in producing false positive reactions in the Captia Syph G EIA. The role of rheumatoid factor (RF) as a potential source of false positives was also analysed.

Methods—Patients who attended a genitourinary medicine (GUM) department and gave a false positive reaction with the EIA between 1988 and 1990 were compared with women undergoing antenatal testing and with the control clinic population (EIA negative) over the same time period. The incidence of sexually transmitted disease (STD) in the clinic population and the false positive reactors was measured in relation to gonorrhoea, chlamydia, genital warts, candidiasis, "other conditions not requiring treatment" and "other conditions requiring treatment." Male:female sex ratios were also compared. Ninety two RF positive sera were analysed with the EIA.

Results—The rate of false positive reactions did not differ with respect to the diagnosis within the GUM clinic population. The antenatal group of women, however, had a lower incidence of false positive reactions than the GUM clinic group. No RF positive sera were positive on Captia Syph G EIA testing.

Conclusions—There is no cross reaction between Captia Syph G EIA and any specific STD or with RF positive sera. The lower incidence of false positive reactions in antenatal women is unexplained but may be related to physiological changes associated with pregnancy.

Introduction

The recent introduction of the Captia Enzyme Immunoassay (Captia Syph G) to detect immunoglobulin G (IgG) antibodies against *Treponema pallidum* presents an opportunity for automated processing and reporting for large numbers of specimens.¹⁻³ This EIA test with an overall sensitivity of over 98% and specificity of over 99%^{2,3} is comparable in performance with the traditional combination of VDRL (Venereal Disease Research Laboratory) and TPHA (*Treponema pallidum* Haemagglutination Assay) tests even in primary syphilis.¹ False negatives in the EIA were associated specifically with primary infection where the sensitivity was 82%.² False positive reactions were not associated with conventional Biological False Positive Reactors.³

The incidence of syphilis in general, and the primary stage in particular, is low in the United Kingdom, however, and the occurrence of false positive reactions, albeit at a low level, decreases the positive predictive value of a positive test. The factors responsible for these erroneous reactions with the EIA are not known.

The antigen in the Captia Syph G EIA is a sonicate of *T. pallidum* (Nichols strain) coated onto microtitration wells. Unlike other *T. pallidum* antigen tests such as the Fluorescent Treponemal Antibody Absorbed (FTA-ABS) test⁴ and the TPHA⁵ that comprise an absorption stage to neutralise group treponemal antibody, the EIA involves no such absorption and relies on serum dilution (1 in 20) to overcome interference from group treponemal antibody.

Because of the lack of an absorption stage it is possible that more false positive reactions might occur in patients attending an STD clinic; this could result from damage to the mucosal epithelium encouraging the growth of commensal treponemes. Excessive growth of such treponemes in genital lesions may give rise to false positive FTA-ABS reactions if the group reactive antibody is not completely neutralised.⁶ In view of this we compared the rate of false positive EIA reactions in patients attending an STD clinic with that for women attending an antenatal clinic. A number of sera positive for rheumatoid factor (RF) were also tested in order to exclude this as a cause of false positive reactions.

Department of Genitourinary Medicine, Edinburgh Royal Infirmary
J Ross, A McMillan

Department of Medical Microbiology, University of Edinburgh
A Moyes, H Young

Table 1 STD prevalence in EIA negative and EIA false-positive clinic population

	Total STD clinic population		False positive EIA STD clinic population	
	<i>n</i> = 12 842		<i>n</i> = 197	
	Male	Female	Male	Female
Gonorrhoea	381	243	5 (1.3%)	2 (0.8%)
Chlamydial infection	2 309	606	31 (1.3%)	8 (1.3%)
Genital warts	2 615	1 893	31 (1.2%)	27 (1.4%)
Candidiasis	88	1 270	0 (0%)	14 (1.1%)
Other (untreated)	2 523	1 544	37 (1.5%)	22 (1.4%)
Other (treated)	2 949	2 529	38 (1.3%)	36 (1.4%)
Pregnancy		10 314		82 (0.8%)

Figures in parenthesis represent the percentage of total incidence for each condition.

Methods

All patients who attended the Department of Genitourinary Medicine, Edinburgh Royal Infirmary between January 1988 and December 1990 and were screened for syphilis by the Captia Syph G (Mercia Diagnostics UK) EIA were included in the study. All sera were heat inactivated at 56°C for 30 minutes prior to serological testing. Sera giving an antibody index³ greater than or equal to 0.9 were repeat tested once by the EIA and were examined by the VDRL, TPHA and FTA-ABS and an EIA for specific antitreponemal IgM (Captia Syph M). Whenever requested, sera from patients at high risk for syphilis, particularly early primary syphilis were examined by all of the above tests. A false positive EIA was taken as an antibody index of greater than or equal to 0.9 which persisted on one repeat test but was not supported by confirmatory treponemal tests and signs or symptoms of treponemal infection.

Comparison was made between the patients with false positive reactions and the overall clinic population with regard to the prevalence of gonorrhoea, chlamydial infection, genital warts, candidiasis, "other conditions not requiring treatment" and "other conditions which required treatment." The last group included patients who had infected partners and received therapy on an epidemiological basis. Patients who had more than one diagnosis were counted in each diagnostic group separately. The male to female sex ratio (M:F) was calculated for each group.

The overall false positive rate in the clinic population was also compared with that of pregnant women screened for syphilis at the Simpson Memorial Maternity Pavilion, Edinburgh between 1988 and 1990.

Sera found positive on screening with the Serascan Rheumatoid Arthritis Latex test (Hycor) were also tested with the Captia Syph G EIA.

Statistical analysis was performed using the chi square test on the Minitab PC statistics package.

Results

Over the 3 year period 12 842 new patients were seen in the Department of Genitourinary Medicine. False positive reactions occurred in 197 (1.5%) compared with 117 confirmed positive tests for syphilis, giving a positive predictive value of 37%. The M:F ratio was 124:73 (1.7:1) in the false positive group compared with 7 593:5 249 (1.4:1) in the STD clinic population, which is not statistically significant. The prevalence of sexually transmitted diseases in the clinic population and in those giving false positive reactions are shown in table 1. The only statistically significant difference between the groups is a lower incidence of genital warts in those with false positive EIA ($p = 0.02$).

Of patients who had positive EIA results 182 (1.4%) became negative on repeat testing.

Antenatal patients (10 314) were tested with 82 false positives (0.8%). This level is significantly lower than the STD clinic population ($p < 0.01$). Only three (1.5%) of the 197 GUM patients and 1 (1.2%) of the antenatal patients with false positive IgG reactions also gave a false positive IgM test.

The RF titres of the RF positive sera are shown in table 2. All 92 of the RF positive sera were negative on testing with the Captia Syph G EIA.

Discussion

Despite a specificity of greater than 99% a positive

Table 2 RF titres in RF positive sera

RF titre (IU/ml)	No of patients
20	38
40	23
80	15
160	6
320	4
640	3
1 280	2
2 560	1
Total	92

IgG EIA, in the population studied, has a positive predictive value of only 37%. This is due to the low prevalence of syphilis and the large number of screening tests performed. Clearly, however, a positive EIA reaction must be confirmed by other treponemal tests. It is essential that laboratories that do not have confirmatory tests on site send EIA reactive sera to a reference laboratory for confirmation.

The incidence of positive tests with the EIA that are unconfirmed (1.4%) is comparable to that with using VDRL and TPHA as screening tests (1.6%).³

Our results indicate that false positive EIA reactions are commoner in an STD population when compared with antenatal women but are not associated with any individual STD. The lower incidence of genital warts in patients with false positive tests compared with the control clinic population is difficult to explain. Warts are controlled mainly via cell mediated immunity and humoral factors are thought to be relatively unimportant.⁷

There were fewer women in the false positive reactor group than the clinic population as a whole but this was not statistically significant. In this context it is interesting that the incidence of false positive IgG EIA results was lower in the group of pregnant women studied compared with the GUM population ($p < 0.01$). Since pregnant women are generally in more stable relationships than the GUM clinic population and are therefore less likely to have genital infections this may indicate that STDs do contribute to false positive reactions. Many other physiological changes in immunity and hormones also occur in pregnancy however and it is unclear as to the relative contribution of these different factors.

Women have a higher incidence of autoimmune diseases in general⁸ but this may not be an important factor in determining a serological false positive reaction in this assay in view of the sex ratio observed in the false positive patients.

Rheumatoid factor, usually in the form of immunoglobulin M (IgM) directed against host IgG, is well recognised in syphilis and increases in incidence in the latter stages of the disease.⁹ The IgM-IgG complex may produce a false positive reaction by mimicking IgM specific antibody in EIA reactions. The absence of any false positive reactions would indicate that rheumatoid factor is not important in producing false positive reactions in the Captia Syph G EIA system, however. RF would also not appear to be a problem in the IgM EIA as evidenced by the very low number of false positive IgM reactions.

It is also possible that false positive reactions may occur owing to the presence of heat shock proteins¹⁰ and endoflagella present in the sonicate of *Treponema pallidum*^{11,12}

In conclusion, no individual sexually transmitted disease is associated with false positive reactions in the Captia Syph G EIA although false reactivity is commoner in an STD population than in pregnant women. This may suggest that the cause of these reactions is multifactorial or due to a different mechanism. Despite a low positive predictive value the test is comparable to traditional screening methods with regard to sensitivity and specificity but is potentially cheaper when large numbers of samples are processed, particularly by automated systems.

Address for correspondence: Dr JDC Ross, Department of Genitourinary Medicine, Edinburgh Royal Infirmary, Lauriston Place, Edinburgh EH3 9YW.

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